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by

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16. ABSTRACT

The potential environmental and health impact of PCBs must account not only for the integrated quantitation of the PCBs mixtures but also the concentrations of the specific congeners which are potentially toxic. Our results confirm the feasibility of high resolution PCB analysis of commercial and environmental PCB mixtures. We anticipate using this approach to accurately measure the concentrations of the specific PCB isomers in environmental and biological samples and to determine the effects of PCB structures on the ecological dynamics of this complex group of chemicals.

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CHAPTER 1

HIGH RESOLUTION PCB ANALYSIS

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HALOGENATED AROMATICS

Polychlorinated biphenyls (PCBs) are members of a group of chemical pollutants generally referred to as the halogenated aromatics. These chemicals include the polychlorinated naphthalenes (PCNs), polychlorinated benzenes (PBs), polychlorinated terphenyls (PCTs) and polybrominated biphenyls (PBBs) which are all primary industrial chemicals. Other members of this group of pollutants include the polychlorinated azo and azoxybenzenes (PCABs and PCAOBs respectively) which are formed as by-products in the preparation of chlorinated aniline-derived chemicals and the polychlorinated dibenzofurans (PCDFs) and dibenzo-p-dioxins (PCDDs) which are formed in the synthesis of chlorinated phenol-derived chemicals and in the combustion of organics (1,2). A major problem associated with the analysis and toxicology of

halogenated aromatics is the multiplicity of possible isomers and congeners. For example, the commercial PCBs are highly complex mixtures of individual compounds whose identities have not been unequivocally determined (1,3-5). Moreover, the composition of PCB mixtures which have been identified in environmental samples is different from that of the commercial mixtures (5-8). This can be attributed to numerous factors such as preferential bio-and photodegradation of specific PCBs and the preferential uptake and retention of individual PCBs in different environmental matrices. For example, compared to commercial PCB formulations, atmospheric PCB residues are enriched in the more volatile lower chlorinated congeners (9); the PCBs which persist in fish extracts tend to be the more highly chlorinated congeners; PCB residues in humans are more highly chlorinated and enriched in PCBs which are not readily metabolized (5-7). Moreover, PCB residues in humans contain a lower percentage of the more highly ortho-chloro substituted isomers and congeners compared to the commercial PCB formulations (5). It is also apparent that the toxicity of PCBs, in common with other halogenated aromatics, is dependent on the structure of the individual compounds. Thus the potential environmental and health impact of PCBs must account not only for the integrated quantitation of the PCBs mixtures but also the concentrations of the specific congeners which are potentially toxic.

PCBs: IDENTIFICATION OF THE POTENTIALLY TOXIC CONGENERS

Structure-activity studies within the class of halogenated aromatics suggest that there is a correlation between those compounds which are toxic and those which induce a microsomal cytochrome P-448 dependent monooxygenase enzyme designated aryl hydrocarbon hydroxylase (AHH) (2,12,13,16). Table 2 summarizes all the PCBs which are AHH inducers (2, 16-22). Although the toxicities of these compounds have not been systematically investigated there are numerous reports which confirm the toxic effects elicited by the 3,3',4,4'-tetra-, 3,3',4,4',5-penta-,2,3,3',4,4'-penta-,2,3,4,4',5-penta-, 3,3',4,4',5,5'-hexa- and 2,3,3',4,4',5-hexachlorobiphenyls (2,11-18,23,24). Clearly the environmental concentrations of those PCBs noted in Table 2 should be a major concern for environmental analytical chemists.

PCBs: LOW TO HIGH RESOLUTION GC ANALYSIS

Table 3 summarizes the major approaches to the analysis of PCBs. The two major low and medium resolution techniques utilize packed column separation coupled with detection by an electron capture (EC) detector or mass spectrometry (MS).

The results then are quantitated by peak or pattern matching with the commercial formulations or known mixtures of these formulations (1,25,26). These low resolution methods can be carried out in most analytical laboratories and provide semi-quantitative integrated concentrations of PCBs in environmental samples. The disadvantages of low and medium resolution PCB analysis include; difficulties in quantitation of PCB residues which have undergone extensive degradation, the lack of quantitative data on individual isomers and the lack of quantitative data on the potentially toxic PCBs.

TABLE 1. Halogenated Aromatics: Multiplicity of Isomers and Congeners

Halogenated Aromatic	PCBs	PBBs	PCABs	PCNs	PCDDs	PCDFs
No. of Congeners	209	209	209	135	75	135

TABLE 2. A Summary of PCB Congeners Which Induce AHH Activity.

Number of Chlorine Substituents and Substitution Pattern			
Cl_4	Cl_5	Cl_6	Cl_7
3,3',4,4'	3,3',4,4',5'	3,3',4,4',5,5'	2,3,3',4,4',5,5'
3,4,4',5	2,3,3',4,4'	2,3,3',4,4',5	2,2',3,3',4,4',5
	2,3',4,4',5	2,3,3',4,4',5'	
	2,3,4,4',5	2,3,4,4',5,6	
	2',3,4,4',5	2,3,3',4,4',6	
		2,2',3,3',4,4'	
		2,2',3,4,4',5'	

High resolution isomer-specific PCB analysis (4,10,27-30) is now a viable analytical procedure. The method incorporates a high resolution glass capillary column separation technique coupled with conventional EC or MS detection and requires all 209 PCB reference standards for preliminary calibration and quantitation. Although the synthesis of the standards is a prodigous task we now can report considerable progress in this area (30). Table 4 summarizes our current status in which

182 of the 209 congeners have been prepared using the convential Cadogan coupling (31) of a chlorinated aniline with a chlorinated benzene (excess) in the presence of amyl nitrite (Scheme 1). The crude PCB reaction products are purified to give single congeners or defined mixtures which are characterized by their proton magnetic resonance (PMR) and mass spectra (30).

TABLE 3. Characteristics of Low, Medium and High Resolution PCB Analysis

Separation	Detection	Quantitation
Packed Column	EC	[a]Peak(s) or Pattern Matching
Packed Column	MS	[b]Peak(s) or Pattern Matching
Capillary Column	EC and MS	[c]Isomer-Congener Response

[a]low, [b]medium and [c]high

TABLE 4. Synthesis of PCB Congeners - Progress

No. of Cl	1	2	3	4	5	6	7	8	9	10
No. of Congeners	3	12	24	42	46	42	24	12	3	1
No. Synthesized or Available	3	9	8	39	42	41	24	12	3	1

HIGH RESOLUTION ISOMER - SPECIFIC ANALYSIS OF COMMERCIAL AND ENVIRONMENTAL PCB MIXTURES

The proposed numbering scheme (4) denotes specific PCB isomers and congeners. Using the synthetic PCB standards, their relative retention times and molar response factors were determined. Octachloronaphthalene (OCN) was used as the reference standard for determining relative retention times and molar response factors.

Analysis of a number of PCB mixtures was determined using a Varian 3700 gas chromatograph equipped with an EC detector and a 50 M fused silica column coated with SE-54. The column temperature conditions were; initial temperature,

100°C; final temperature, 240°C; temperature program, 1° min^{-1}. The high resolution gas chromatographic separation of Aroclor 1260, Aroclor 1254, Aroclor 1016 and a mixture of Aroclors 1260 plus 1254 plus 1016 (2:3:5 by weight) are displayed in Figures 1,2,3 and 4 respectively. Based on the relative retention times of the synthetic PCB standards the identities of many of the GC peaks has been assigned. Figure 5 illustrates the more complex gas chromatograph of a cleaned-up PCB fraction obtained from a fish extract. A more comprehensive structure assignment of the GC peaks in this sample will require further GC-MS analysis to identify the chemical structures of the highly complex coextractives.

Our results thus confirm the feasibility of high resolution PCB analysis of the commercial and environmental PCB mixtures. We anticipate using this approach to accurately measure the concentrations of the specific PCB isomers in environmental and biological samples and to determine the effects of PCB structures on the ecological dynamics of this complex group of chemicals.

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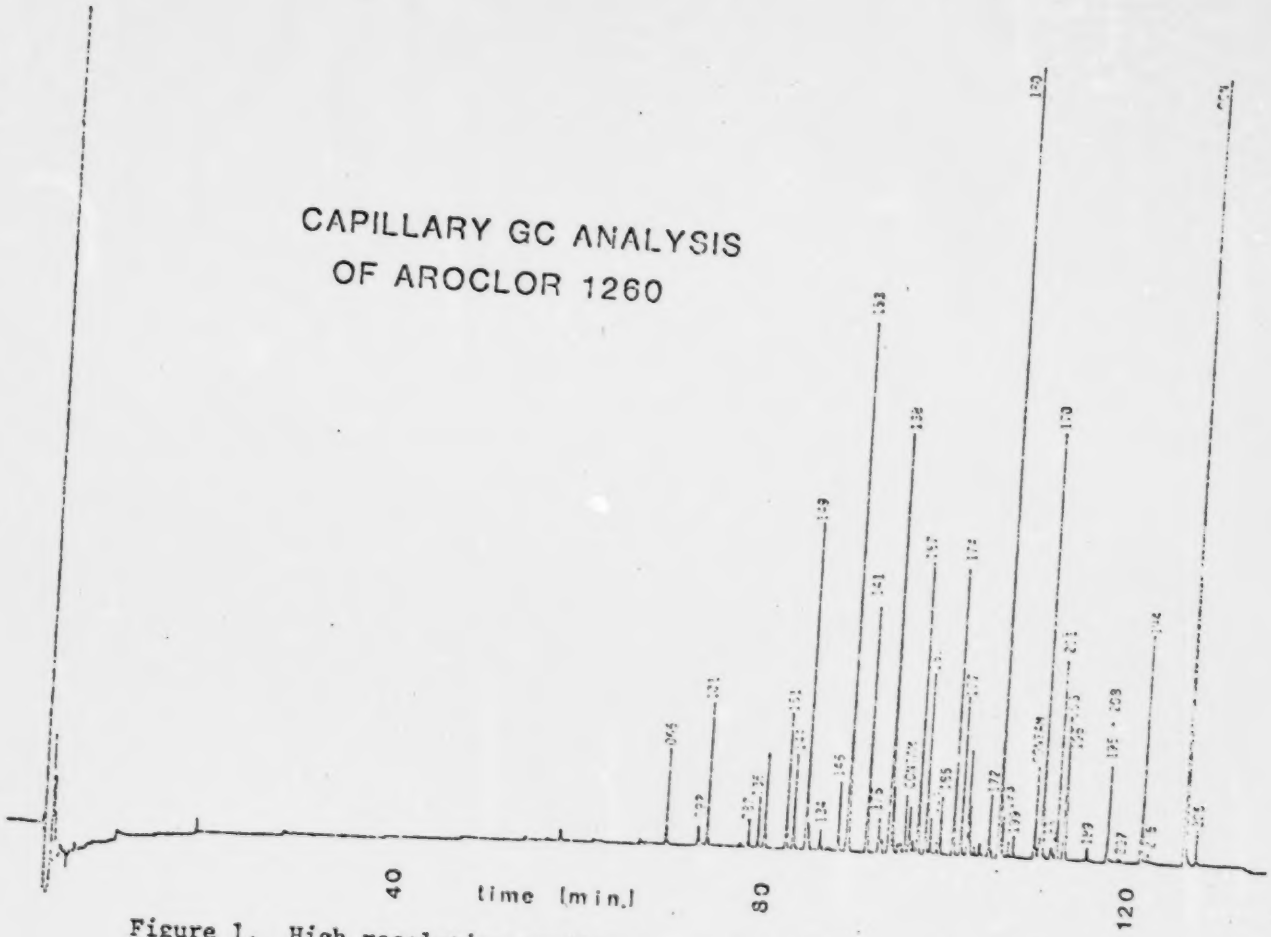

Figure 1. High resolution capillary gas chromatogram of Aroclor 1260

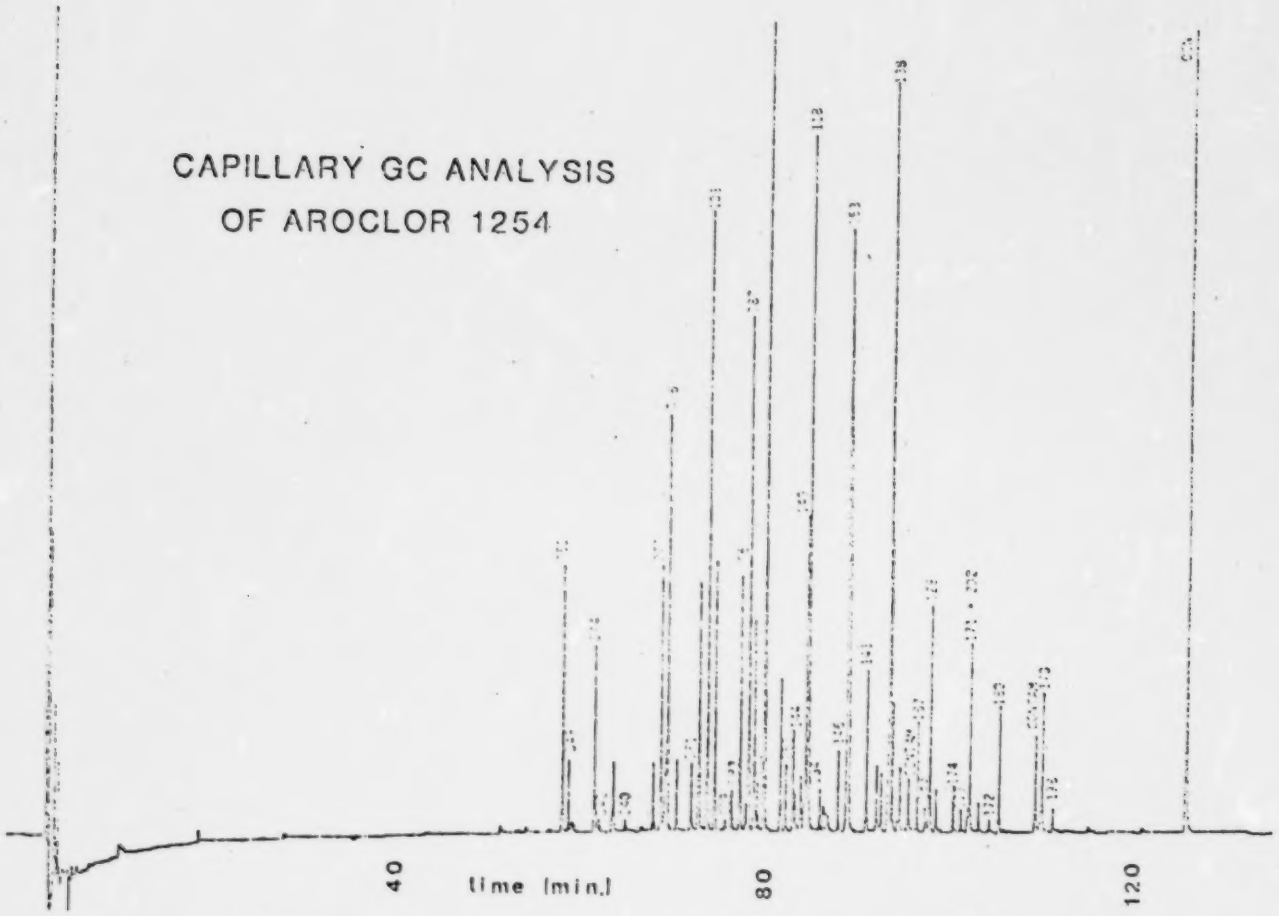

Figure 2. High resolution capillary gas chromatogram of Aroclor 1254

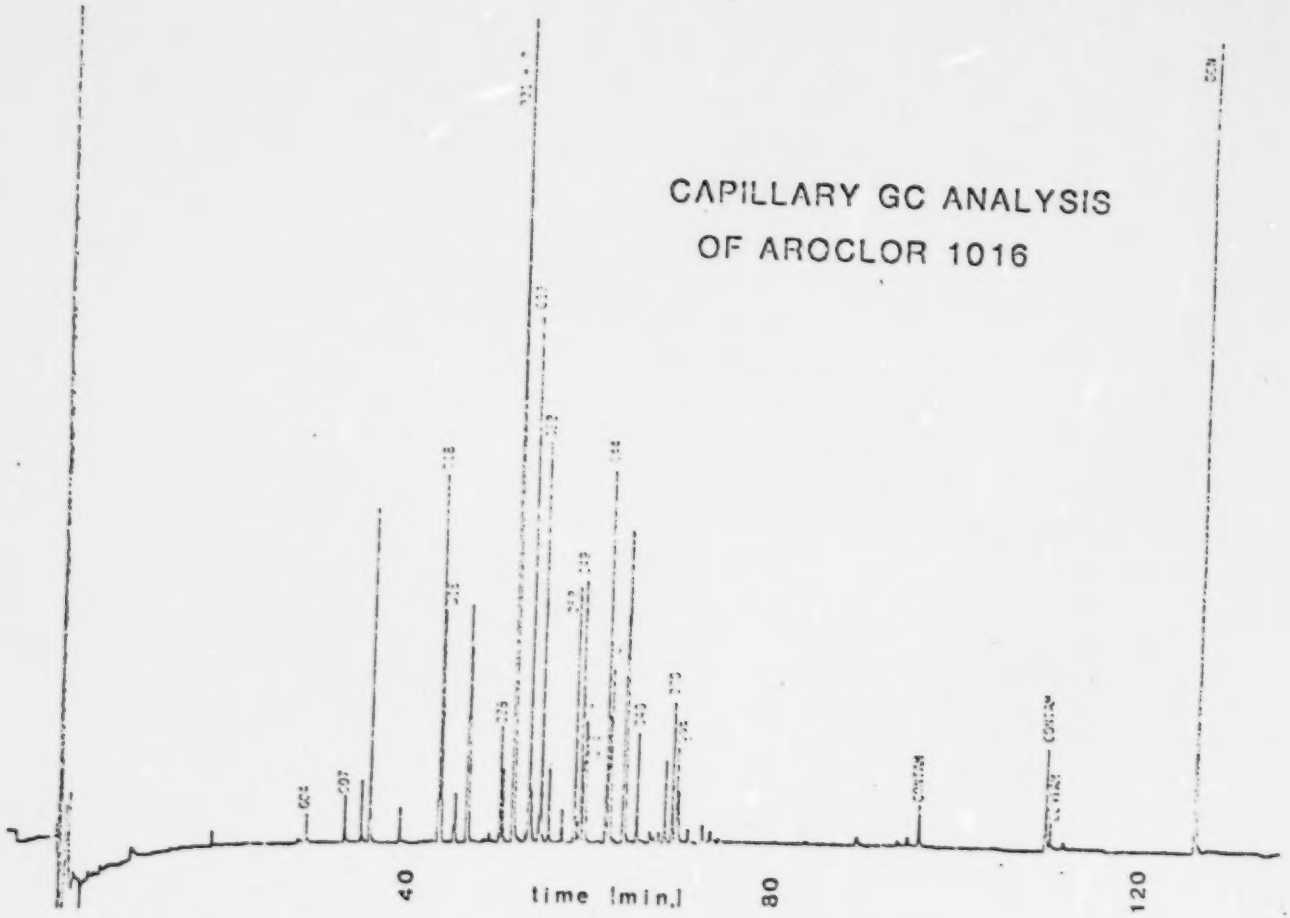

Figure 3. High resolution capillary gas chromatogram of Aroclor 1016

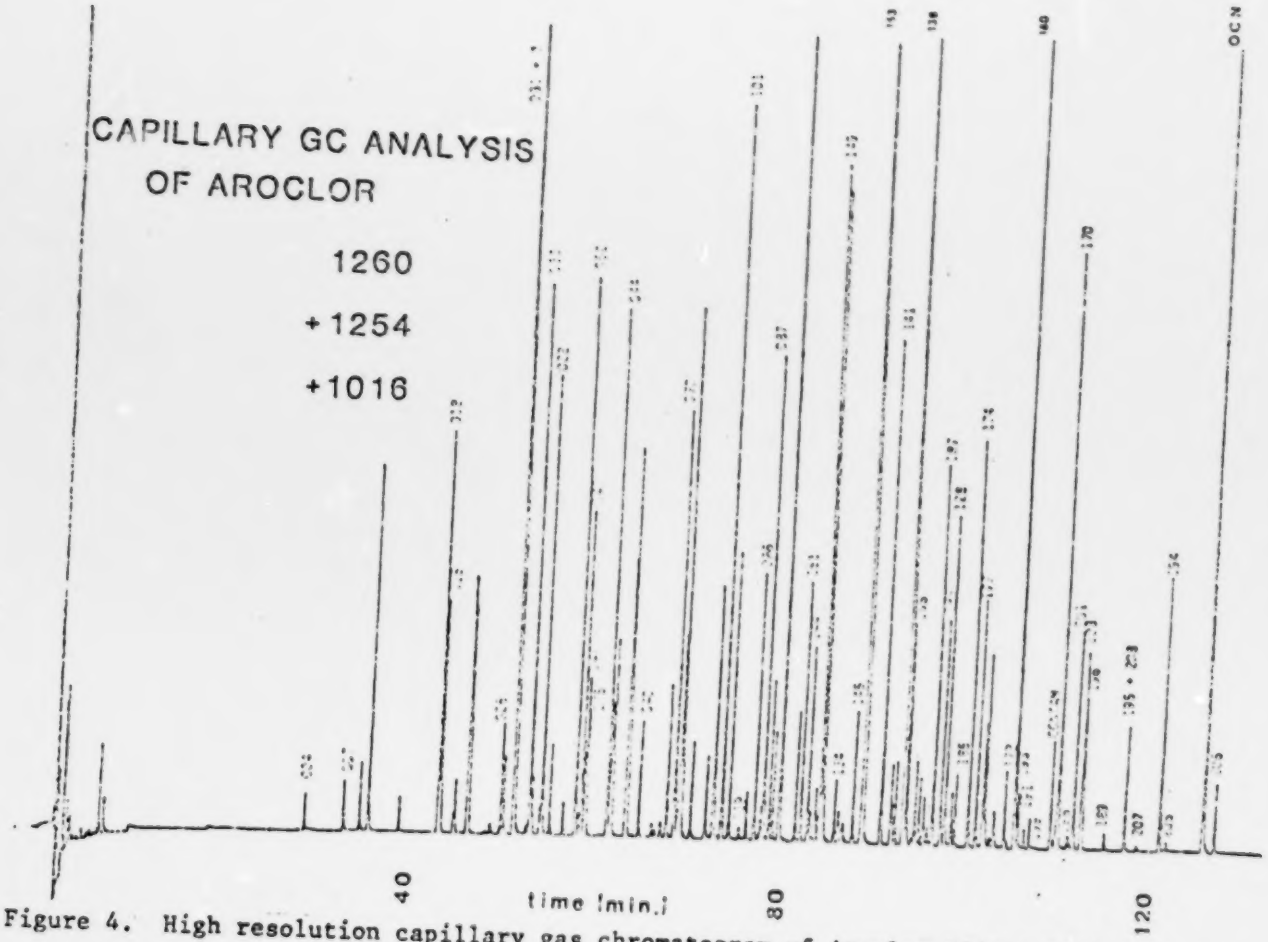

Figure 4. High resolution capillary gas chromatogram of Aroclor 1260 + 1254 + 1016

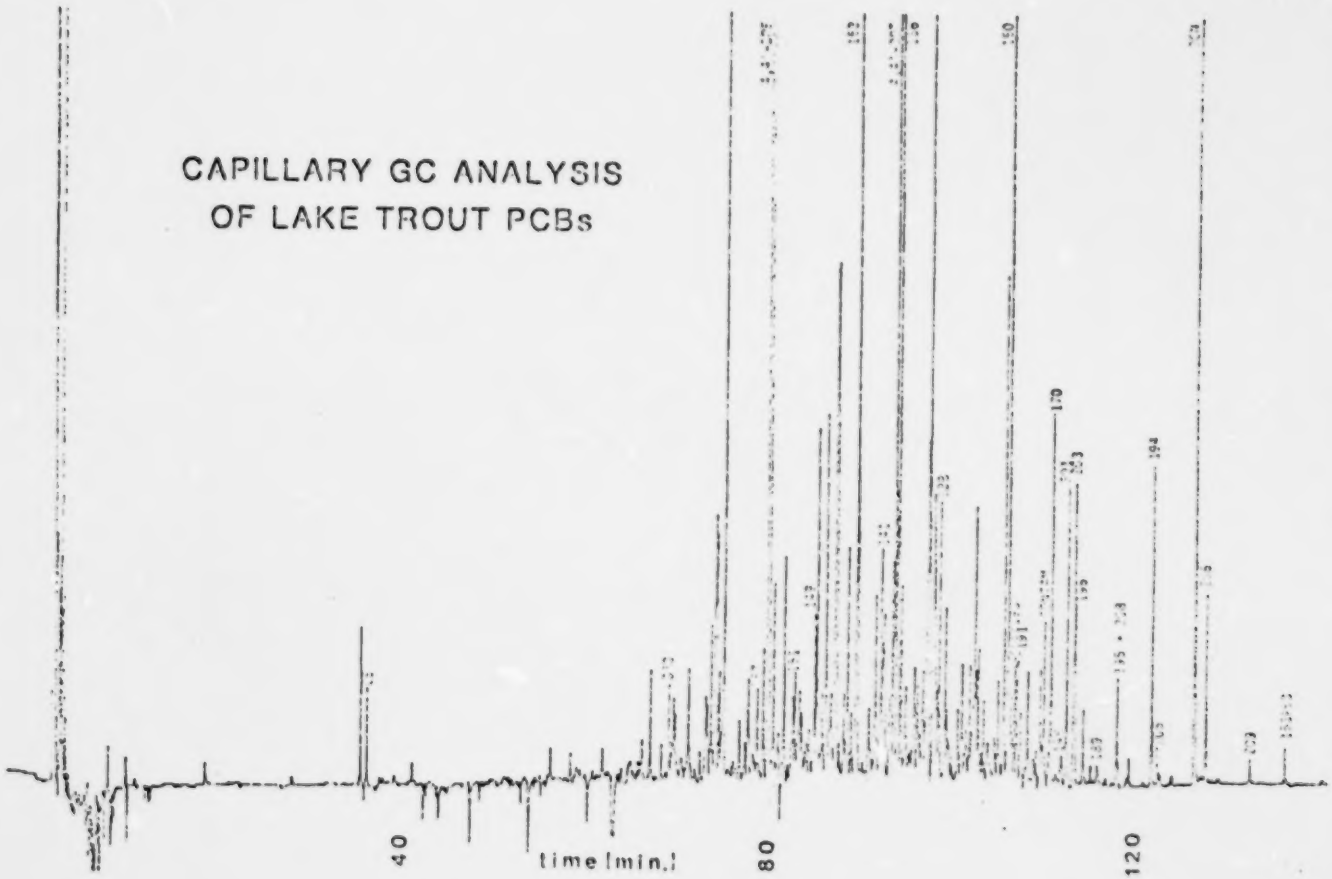

Figure 5. High resolution capillary gas chromatograph of a PCB fraction from a fish extract